Progress Report – FY2005

Core Name: Pathogen Source Tracking

Project Title: Harmful Algal Bloom Identification Tools

Reporting Period: October 1, 2005 - September 30, 2006

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Background and Rationale:

Over the last five years, an unprecedented density and prevalence of harmful algal blooms (HABs) have been found in South Carolina brackish stormwater detention ponds, along with an anomalous raphidophyte algae bloom extending to coastal waters. All of the observed HABs have precedence for toxicity and causing fish kills, including *Pfiesteria* spp., *Heterosigma akashiwo, Chattonella verruculosa, C. subsalsa, Fibrocapsa japonica, Prymnesium parvum,* and *Karlodinium micrum*. HAB species have been associated with 97 of 122 fish kills from 2001-2005, with 25 involving a bloom. Ten of these blooms were also associated with high dissolved oxygen, indicating a strong possibility of toxic effects. Evidence for HAB toxicity to fish was obtained in two of the fish kills including a *Pfiesteria*-linked fish kill in Hilton Head Island and a *K. micrum*-associated fish kill in Charleston. Five of the *Chattonella* or *Fibrocapsa* blooms were positive for the neurotoxin brevetoxin or a brevetoxin-like substance. All of these species have precedence for causing major fish kills around the world, and brevetoxin can cause neurotoxic shellfish poisoning and human respiratory problems. In addition to their potential environmental or human health effects within or near pond boundaries, tidal transport of harmful algal cells, cysts, or toxins may adversely affect fish or shellfish in adjacent tidal creeks or open estuaries.

The harmful algae blooms in retention ponds, adjacent tidal creeks, and open estuaries are a potential threat to fish, shellfish, and humans. Unfortunately, at the moment, identification of harmful algal species for these environments is time consuming and cumbersome. Currently the only way to identify many of these harmful algal species is either by microscopic examination (which requires a trained expert) or real-time PCR (which can only test for one species at a time and takes 4 hours). A real need exists for a single assay that can rapidly and accurately identify multiple harmful algal species simultaneously. The goal of this project is to address that need.

Objectives:

- To develop molecular-based assays for the rapid, parallel detection of HAB species common to Atlantic and Gulf of Mexico coastal waters.

- To create an economical version of the technology that will enable transfer to agencies with limited throughput and/or limited budgets.

- To create a high-throughput robotic version of the technology geared toward large research laboratories capable of examining hundreds of samples per hour.

Accomplishments to date:

- **Development of SIVCA (species identification via chimeric amplification), a novel two-stage Multiplex Polymerase Chain Reaction (PCR) protocol.** The polymerase chain reaction (PCR) is a common tool used by molecular biologists to selectively amplify specific genes (or pieces of genes) from a mixed pool of DNA. This new method of PCR involves identification and labeling of multiple targets in the first step and amplification and addition of a fluorescent dye in the second. This new technique for multiplexing and fluorescently labeling target DNA provides two major benefits: 1) by varying the amount of each first stage primer, differences in reaction efficiency are compensated for, and 2) a single fluorescent label can be used to label all products resulting in dramatic cost savings. This new technique will be useful not only to OHHI and NOAA scientists, but will also provide a new paradigm for simultaneous multiplexing and fluorescent labeling of PCR products by other scientists. One of the difficulties in multiplex PCR is differences in efficiency from one target to another. SIVCA allows us to compensate for those differences and amplify many target species in a single sample. Current PCR assays for algal identification target a single species, but our two-stage multiplex can identify 12 species in only 2 reactions! Already we are increasing the potential throughput by ten fold.

- **Development of a low-cost version of the assay.** A key corollary to the success of the project is the ability for users with varying levels of technology and funds to have an assay that will meet their needs. We designed the assay such that all of the products are distinguishable from one another by size (molecular weight) alone. This means that a small lab with minimal resources can perform these analyses quickly and reliably, albeit with lower throughput. Using relatively inexpensive (and reusable) high-density agarose we can distinguish all products in each of the reactions. This requires neither specialized equipment (apart from what is normally present in a molecular biology lab) nor the expensive fluorescent primer, resulting in a very low-cost assay. Increased accuracy can be accomplished with a minimal increase in price by use of acrylamide electrophoresis.

- **Development of an automated high-throughput detection assay.** Using SIVCA we have been able to attach a fluorescent dye to all products amplified in our reactions. Due to the difference in size of each product, we can detect and identify each product using a commercially available capillary electrophoresis system. This automation allows us to screen up to 96 samples at a time, all in a

matter of hours. To screen that many samples for 12 species would require at least eleven days with currently available real-time PCR protocols.

Expansion of the assay. During FY 2005 the assay was expanded to amplify 12 species of harmful algae. The species targeted are some of the most common in on the eastern US coast and were chosen for their frequency, their difficulty identify positively, or both. These include: *Aureococcus annophagefferens, Chattonella marina, Chattonella subsalsa, Heterosigma akashiwo, Fibrocapsa japonica, Microcystis aeruginosa, Karlodinium micrum, Kryptoperidinium foliaceum, Pfiesteria piscicida, Pfiesteria shumwayae, Prorocentrum minimum,* and *Pseudo-nitzschia seriata*. The assay is being optimized to limit run time by determining the fastest it can be run with acceptable results. The assay volume is being optimized to use the smallest amounts of reagents necessary to achieve quality results. Finally the reaction temperatures and times are being optimized to increase efficiency and lower detecton limits.

Field Validation of the Assay. During the summer of 2006, samples collected during routine monitoring by the South Carolina Harmful Algal Bloom Program that contained any of the species of interest were archived. These samples, stored on glass fiber filters and stored in buffer at -20°C are currently being assessed with both SIVCA and conventional real-time PCR to determine the effectiveness of SIVCA in real field samples. Analysis of these samples will begin in November, 2006.

Presentations:

Brown, P.J.P., Chapman, R., and Lewitus, A. 2005 Two step chimeric multiplex fluorescent PCR as a means of detecting harmful algal species from the environment. Invited presentation at the Estuarine Research Foundation's special session on Oceans and Human Health. ERF 2005 Norfolk, Virginia USA.

Brown, P.J.P., Chapman, R., and Lewitus, A. 2006 Species Identification via Chimeric Amplification: A novel technique for simultaneous detection of multiple harmful algal species. Podium presentation at the Oceans and Human Health Initiative all-PI meeting, Charleston, SC.

Publications:

Brown, P.J.P, Lewitus, A.J., Wilde, S.B. and Chapman, R. 2007 Species Identification via Chimeric Amplification: A novel technique for simultaneous detection of multiple harmful algal species. Manuscript in preparation for submission to *Nature: Methods* in early 2007.

Application/Technology Transfer relevant to OHH Strategic Goals

1.0 Scientific Research and Application

The technology that we are developing will enable researchers to take a snapshot of the harmful algal population in a given body of water. The throughput capability and ease of use of this technology will provide a valuable new tool to researchers interested in understanding changes in algal species composition in a variety of water bodies. This technology is also expandable to other groups of organisms (e.g. enteric bacteria) and can be scaled up or down depending on the needs of the researcher. Potential targets include those bacteria involved in a particular ecological process (such as denitrification), enteric bacteria from human and non-human sources, or even a group of the most common bioterrorism agents. We have essentially developed ground-level technology that has myriad potential applications.

2.0 Public Information and Outreach

One of the major benefits of our HAB identification tool is its usefulness to natural resource and land management officials. The decision to close a beach or a shell fish bed due to an algal bloom can have exceptional financial implications. A resource manager doesn't want to make that kind of decision without all the facts. There are several algal species that are similar to HAB species but are not themselves toxic. Our tool rapidly and accurately identifies many potentially harmful species while excluding these non-toxic look-alikes. While it would be ideal to only base these decisions on whether or not a bloom was toxic, the toxic mechanisms for many of these organisms have not been determined. Additionally, until more is known about the environmental triggers of algal toxicity, prudence demands that decisions be made based on the presence of the organism, not whether or not its toxic at the moment. This enables decision makers to make rapid and informed decisions regarding closure of commercial and recreational resources, thus protecting human health without endangering commerce. Due to the scalability of this tool, the low-cost option will be available to state agencies and many local municipalities, enabling local officials to make public health decision regarding HABs quickly and for the common good.

Project abstract:

The goal of the harmful algal identification tools project is to create a single DNA-based test that can screen for the presence of the most problematic algal species on the eastern seaboard. Harmful algal blooms (HABs) can cause extensive damage to fisheries, recreational waterways, and human health. Knowing exactly which harmful algal species (if any) are present during a bloom event (noticeable discoloration of the water) is paramount to effective decision making by resource managers. Current technologies require a trained expert, expensive and time consuming analyses, or both. We are creating a single tool that can be implemented by a variety of users to rapidly and accurately identify multiple harmful algal bloom (HAB) forming species. Since currently available technologies are either too cumbersome or cost prohibitive to meet our needs, we have developed an entirely new technology. Species Identification via Chimeric Amplification (SIVCA) uses a combination of short DNA primers to selectively amplify specific genes from different harmful algal species. We have successfully developed and implemented probe sets targeted to 12 different species. In addition, we have used this novel method of gene amplification to generate two detection methods, one that is easily

accessible and low cost; the other being more expensive but with dramatically higher throughput. We plan to bring the total number of species in the assay up to 18 and to transfer the reactions over to robotic equipment, further increasing our automation and throughput. Even at its current developmental stage, SIVCA has the potential to revolutionize the way multiple species are detected and can have broad implications in not only environmental health, but also in ecology and even bioterrorism.

Unresolved Issues:

- Although this assay will eventually be automated using robotics, we have yet to adapt it to the robot. This has been mainly due to time constraints on P. Brown. Therefore, the ease of transferring the assay over to the robot has not been determined.

Budget Report:

Our budget includes salary and fringe for a Biologist III at SCDNR ($52,492 in year one, $54,068 in year two, $26,737 for 6 months of year three). All supplies have been purchased through the HML (Marine Genomics Core), as all aspects of this project have been performed in tight collaboration with them.